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Varicella-Zoster Virus Gene Expression in Latently Infected and Explanted Human Ganglia

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A consistent feature of varicella-zoster virus (VZV) latency is the restricted pattern of viral gene expression in human ganglionic tissues. To understand further the significance of this gene restriction, we used in situ hybridization (ISH) to detect the frequency of RNA expression for nine VZV genes in trigeminal ganglia (TG) from 35 human subjects, including 18 who were human immunodeficiency virus (HIV) positive. RNA for VZV gene 21 was detected in 7 of 11 normal and 6 of 10 HIV-positive subjects, RNA for gene 29 was detected in 5 of 14 normal and 11 of 11 HIV-positive subjects, RNA for gene 62 was detected in 4 of 10 normal and 6 of 9 HIV-positive subjects, and RNA for gene 63 was detected in 8 of 17 normal and 12 of 15 HIV-positive subjects. RNA for VZV gene 4 was detected in 2 of 13 normal and 4 of 9 HIV-positive subjects, and RNA for gene 18 was detected in 4 of 15 normal and 5 of 15 HIV-positive subjects. By contrast, RNAs for VZV genes 28, 40, and 61 were rarely or never detected. In addition, immunocytochemical analysis detected the presence of VZV gene 63-encoded protein in five normal and four HIV-positive subjects. VZV RNA was also analyzed in explanted fresh human TG and dorsal root ganglia from five normal human subjects over a period of up to 11 days in culture. We found a very different pattern of gene expression in these explants, with transcripts for VZV genes 18, 28, 29, 40, and 63 all frequently detected, presumably as a result of viral reactivation. Taken together, these data provide further support for the notion of significant and restricted viral gene expression in VZV latency.

Several laboratories over the last decade have used in situ hybridization (ISH) and/or PCR amplification in attempts to define unambiguously the physical state of latent varicella-zoster virus (VZV), including the cell type specificity of latent virus, since this has important implications for viral neuropathogenesis (8, 18). The cell type specificity of latent VZV had been controversial for many years, with some investigators reporting an exclusively neuronal location (7, 9) and others reporting a nonneuronal site (5, 19), while latent VZV had also been reported to be present in both neuronal and satellite cells (15). Clarification of this issue was provided by two recent studies, one of which used ISH and PCR amplification to show that latent VZV in human trigeminal ganglia (TG) was located predominantly in neurons with only occasional satellite cells infected (12), while the other used a dissociated cell technique using neuronal and nonneuronal TG cell fractions to indicate that latent VZV DNA is present primarily, if not exclusively, in neurons (14). A further study demonstrated that, as expected, more than one region of the VZV genome is present in neurons during latency in dorsal root ganglia (DRG) and that both a VZV gene and its corresponding RNA transcript can be present in serial sections in the same region of the ganglion (13).

Transcription during latency of several VZV genes, including genes 21, 29, 62, and 63, has been reported by several groups (1–4, 13, 19), and there is also weaker evidence for transcription of gene 4 and other genes (5, 18, 19). By contrast, the only herpes simplex virus (HSV) transcripts which have been detected are the latency-associated transcripts (21). The significance of the apparently highly restricted pattern of VZV gene expression during latency is not known. There is also some evidence for VZV protein expression in ganglionic tissues during latency in that VZV gene 63-encoded protein has been reported to be expressed in the cytoplasm of neurons in latently infected ganglia (17), and a recent study has also reported the presence in normal ganglia of proteins encoded by all of the VZV genes which are expressed during latency (16).

A major theoretical problem with these RNA detection studies of latently infected human ganglia is that VZV may have reactivated immediately after death and/or removal of ganglionic tissue, making interpretation of putatively expressed VZV genes very difficult. We addressed this issue in the present study by hypothesizing that (i) truly expressed VZV genes in ganglia should be detected more frequently than non-latently associated genes, which may have been present only due to localized viral reactivation, and (ii) the pattern of VZV RNA expression in latently infected ganglia should be different from that in explanted ganglia in which VZV had reactivated in vitro. We provide evidence here that supports these two postulates.

MATERIALS AND METHODS

Human tissue specimens. Human ganglionic autopsy tissues (TG and DRG) were kindly donated by the Medical Research Council HIV Brain and Tissue Bank in Edinburgh, Scotland, S. Straus and R. Kost (Bethesda, Md.), D. Gilden (Denver, Colo.), and D. Doyle and D. Graham (Glasgow, Scotland). We studied a total of 35 subjects (Tables 1 and 2, 18 of whom were infected with human immunodeficiency virus (HIV) (13 with AIDS and 5 with pre-AIDS). To our knowledge, the six subjects with unknown diagnoses in Table 1 were immunocompetent and not HIV positive. In addition, control tissues included were VZV-infected and uninfected CV-1 (African green monkey) cells, VZV-infected human skin, and uninfected rat ganglia. Putative negative controls also included two neonatal (<4-week-old) TG. Samples were fixed in formalin or paraformaldehyde prior to wax embedding. For studies shown in Tables 1 and 2, one ganglion specimen from each subject was available and used. Each tissue sample was studied at least twice for each probe in ISH assays.

Oligonucleotide probes. The probes used in this study (Table 3) included probes for VZV genes 4, 18, 21, 28, 29, 40, 61, 62, and 63. These were chosen as representing genes which had previously been reported to be expressed during

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TABLE 1. Detection by ISH of VZV RNA in normal human TG[a]

Patient	Age	Sex	Diagnosis	Gene 4	Gene 18	Gene 21	Gene 28	Gene 29	Gene 40	Gene 61	Gene 62	Gene 63
1	27 yr	M	OD	−	+	+	ND	ND	−	−	+	+
2	26 yr	M	OD	ND	+	ND	ND	−	ND	ND	ND	+
3	26 yr	M	OD	−	−	+	−	+	−	ND	+	+
4	44 yr	F	Crohn's disease	−	−	+	ND	−	ND	ND	ND	−
5	Adult	?	Unknown	ND	−	ND	ND	+	+	ND	ND	+
6	Adult	?	Unknown	−	−	ND	−	−	ND	ND	−	+
7	22 yr	M	Gunshot wound	+	−	+	ND	+	ND	ND	ND	+
8	Adult	?	Unknown	−	−	+	−	−	ND	ND	−	−
9	Adult	?	Unknown	ND	−	ND	ND	−	ND	ND	ND	−
10	Adult	?	Unknown	±	+	+	ND	+	ND	ND	ND	+
11	63 yr	M	Myocardial infarction	ND	−	ND	ND	−	ND	ND	ND	−
12	29 yr	M	Normal	−	ND	−	−	ND	ND	ND	−	−
13	36 yr	F	Asthma	−	−	−	−	ND	ND	ND	−	−
14	37 yr	M	Gunshot wound	+	+	+	ND	+	ND	−	+	+
15	Adult	?	Unknown	−	−	−	−	±	−	−	−	−
16	30 yr	M	Gunshot wound	−	−	−	ND	−	ND	ND	+	−
17	34 yr	F	Smoke inhalation	−	ND	ND	−	−	−	ND	−	−

[a] M, male; F, female; ND, not determined; ?, unknown; OD, drug overdose; +, VZV positive; −, VZV negative; ±, equivocal result.

VZV latency (genes 4, 21, 29, 62, and 63) as well as those which are not thought to be expressed (genes 18, 28, 40, and 61). A probe control for human β-globin was also used. All oligonucleotides were synthesized by Genosys UK.

ISH. ISH for VZV RNA was carried out as previously described (13). Five-micrometer tissue sections were placed onto glass slides which had been precoated with 3-aminopropyltriethoxysilane, incubated overnight at 37°C, dewaxed in xylene, and then rehydrated through graded ethanols to diethyl pyrocarbonate-treated H_2O. The slides were then treated with 0.2 N HCl and proteinase K. In the case of cultured cells, the HCl procedure was omitted. The proteinase K was then inactivated by treatment with glycine–phosphate-buffered saline PBS, following which the sections were acetylated in triethanolamine-acetic anhydride. The sections were then dehydrated, and prehybridization was performed for at least 2 h in buffer containing formamide, 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 1× Denhardt's solution, denatured salmon sperm DNA, and yeast tRNA. Tissue sections were then hybridized overnight at 37°C with the appropriate digoxigenin (DIG)-labeled probe in dextran sulfate prehybridization buffer. Unbound probe was removed by washes with 2× SSC (twice for 15 min each time), 1× SSC (twice for 15 min each time), and 0.1× SSC (twice for 15 min each time) at 42°C. The resulting hybrids were detected using standard DIG reagents (Boehringer Mannheim). The slides were dipped in buffer 1 (0.1 M maleic acid, 0.15 M NaCl [pH 7.5]) and then exposed for 30 min to buffer 2 (10% DIG blocking agent). After exposure for 60 min to antibody conjugated to alkaline phosphatase which had been diluted in buffer 2, the slides were washed twice for 10 min each time in buffer 1. Sections were then equilibrated in buffer 3 (100 mM Tris-HCl [pH 9.5], 100 mM NaCl, 50 mM $MgCl_2$) and the detection reagent (45 μl of nitroblue tetrazolium [NBT] and 35 μl of 5-bromo-4-chloro-3-indolylphosphate [BCIP] in 10 ml of buffer 3) with levamisole (0.24 mg/ml) and left in the dark for 15 min to 2 h, until development of the purple color. As described before (12, 13) several sections, including controls, were processed on the same slide to avoid bias in the interpretation of color development, and all slides were read blind.

Immunocytochemistry. The tissue sections were dewaxed, rehydrated through graded ethanols to PBS, and then incubated for approximately 1 h in PBS containing 1% bovine serum albumin and 1% normal sheep serum. Rabbit polyclonal antibody (diluted 1:500) to VZV gene 63-encoded protein was a gift from C. Sadzot and B. Rentier (6, 17). The antibody was diluted appropriately in blocking buffer and incubated at 4°C overnight. After returning to room temperature, the slides were washed three times for 5 min each in PBS and then incubated for 1 h in a 1:300 dilution of anti-rabbit antibody (DAKO) in blocking buffer. PBS washes were repeated as above, and the sections were incubated at room temperature in a 1:100 dilution of streptavidin-alkaline phosphatase conjugate (DAKO) in blocking buffer, washed three times in PBS, and then developed using NBT-BCIP or a fuchsin kit (DAKO) containing levamisole (0.24 mg/ml) for 10 min. The slides were then washed in distilled water and mounted using an aqueous mountant.

Explanted TG and DRG experiments. Human ganglia (TG or DRG) from normal subjects were obtained at autopsy 14 to 48 h after death. Each ganglion was then divided into four pieces. One piece was immediately fixed overnight in 4% paraformaldehyde or formalin, and the other three were cultivated as explants on a monolayer of CV-1 cells in RPMI 1640 containing 5% fetal calf serum for 64 to 264 h after death, with typical sampling time points at 64, 120, 168, 192, and 264 h. Cocultivation of explanted ganglion tissues with CV-1 cells

TABLE 2. Detection by ISH of VZV RNA in human TG from HIV-positive subjects[a]

Patient	Age (yr)	Sex	Diagnosis	Gene 4	Gene 18	Gene 21	Gene 28	Gene 29	Gene 40	Gene 61	Gene 62	Gene 63
18	33	M	AIDS	+	ND	ND	ND	+	−	ND	+	+
19	37	M	AIDS	ND	−	ND	ND	+	ND	ND	ND	+
20	28	M	AIDS	−	−	ND	ND	+	ND	ND	ND	+
21	50	M	AIDS	+	+	+	−	ND	ND	+	+	+
22	30	M	AIDS	ND	−	ND	ND	ND	ND	ND	ND	−
23	33	M	AIDS	ND	−	ND	ND	ND	ND	ND	ND	−
24	38	F	AIDS	+	−	−	ND	+	ND	ND	+	+
25	46	M	AIDS	−	−	+	−	+	−	ND	−	+
26	26	M	AIDS	−	−	+	−	+	+	ND	+	+
27	27	M	AIDS	ND	ND	−	−	ND	−	ND	ND	ND
28	39	M	AIDS	−	−	−	−	+	−	ND	−	−
29	37	M	AIDS	+	−	+	ND	ND	ND	−	ND	+
30	25	F	AIDS	ND	+	+	ND	+	ND	−	ND	+
31	29	M	Pre-AIDS	−	ND	+	−	ND	ND	ND	−	ND
32	23	M	Pre-AIDS	ND	+	ND	ND	+	−	−	+	+
33	29	M	Pre-AIDS	ND	+	ND	ND	ND	ND	ND	+	+
34	30	M	Pre-AIDS	ND	+	−	ND	+	−	ND	ND	ND
35	30	M	Pre-AIDS	ND	−	ND	ND	+	−	ND	ND	+

[a] M, male; F, female; ND, not determined; +, VZV positive; −, VZV negative.

TABLE 3. Oligonucleotide probes used in this study

VZV gene	Putative or known function[a]	HSV-1 homolog	Oligonucleotide sequence (5′-3′)
4	Transactivation	UL54 ICP27	TGCAACCTCGAAGTCACTT
18	Ribonucleotide reductase	UL40	GATTCGGACTTTCCACTTGCA
21	Unknown	UL37	GGTCACTCCCACTTGTATTCC
28	DNA polymerase	UL30	CGGAACTTCTTTTCCATTACAGTA
29	Major DNA binding protein	UL29	TCATCTAGAATCTTTACTGCTTCTAGAGCGCCTTC TACGGTCCAGGGCGTTTCCAGGGTTTGGATAATC
40	Major capsid protein	UL19	TCTAGAAAACGCACAAAGTTTAAT
61	Transrepression	ICP0 IE110	TGTGGAGAGAGGCAACTTTGC
62	Transcriptional activation Repression	ICP4 IE175	GAGTTTGTTTCGTCTTCATCCTCT
63	Transcriptional activation Repression	ICP22 IE68	CGCGCTTAAGCTACACGCCATGGGGGGGCGG

[a] See references 3, 10, and 20.

allowed the possible detection of infectious virus release. Ganglion tissue pieces removed from RPMI 1640 at various time points were immediately fixed in 4% paraformaldehyde or formalin, washed in PBS, and frozen in PBS-sucrose at 70°C until they could be wax embedded, sectioned with a microtome, and processed for ISH as described above. All tissues from the same patient were processed and wax embedded at the same time.

RESULTS

Frequency of RNA expression for nine VZV genes in human TG detected by ISH. VZV RNA corresponding to nine viral genes was examined in a total of 35 subjects (Tables 1 and 2); of these, 17 were immunocompetent at the time of death and 18 were HIV infected. The latter were chosen because VZV reactivation might be expected to occur more frequently in HIV-positive individuals (12). The VZV genes studied were chosen as representative genes spanning most of the viral genome; they included both genes which have been reported to be expressed during VZV latency (genes 4, 21, 29, 62, and 63) and genes which have not been previously identified as being expressed during latency (genes 18, 28, 40, and 61). Since this study was limited by the availability of human TG tissues, not all genes could be examined in all cases. It can be seen from Tables 1 and 2 that, even allowing for differences in the numbers of cases studied, RNA for genes 21, 29, 62, and 63 were the most frequently detected in both the normal and HIV-positive subjects. RNA for gene 21 was detected in 7 of 11 normal and 6 of 10 HIV-positive subjects. RNA for gene 29 was detected in 5 of 14 normal and 11 of 11 HIV-positive subjects. RNA for gene 62 was detected in 4 of 10 normal and 6 of 9 HIV-positive subjects. RNA for gene 63 was detected in 8 of 17 normal and 12 of 15 HIV-positive subjects. By contrast, RNA for genes 28, 40, and 61 were never or only rarely detected. Thus, RNA for gene 28 was not detected in any of seven normal or six HIV-positive subjects, RNA for gene 40 was detected in one of five normal and one of eight HIV-positive subjects, and RNA for gene 61 was detected in none of three normal and one of four HIV-positive subjects. The two genes which were detected at an intermediate frequency were genes 4 and 18. Thus, RNA for gene 4 was detected in 2 of 13 normal and 4 of 9 HIV-positive subjects, while RNA for gene 18 was detected in 4 of 15 normal and 5 of 15 HIV-positive subjects. In the few cases where repeated studies gave unclear results, an equivocal result was recorded. In both normal and HIV-positive subjects, the VZV RNA was located predominantly in neuronal nuclei (Fig. 1), with only occasional (<0.1%) positive nonneuronal cells. Analysis of the HIV-positive and normal cases (with an average neuron count of 72 cells per slide) carried out on representative sections from several subjects with four to six different probes showed that the proportions of neurons which were viral RNA positive were approximately 7% for the normal and 5% for the HIV-positive cases. However, the very variable and focal distribution of the RNA-positive neurons makes these only estimated figures. The sample size and variability did not allow formal statistical analysis to be carried out, either for the latter cell counts or for the relative frequencies of the different RNA-positive cases. No RNA signal was detected in this study in the negative control tissues such as the neonatal human TG or uninfected rat ganglia, while VZV-infected cells were positive (Fig. 1). To check that cellular RNA was intact in these TG tissues, ISH was also performed at least once for each sample with a human β-globin probe and was found to be positive (data not shown).

Presence of VZV gene 63-encoded protein in TG neurons. Since we had previously reported the presence of DNA for VZV gene 63 in human TG (12), and in view of the detection of RNA for this gene described above, as well as previous reports of gene 63-encoded protein by others (17), we carried out immunocytochemistry to locate this protein in both normal and HIV-positive subjects. Only a minority of samples from both patient groups were positive when TG sections were labeled with the anti-63 protein antibody (Fig. 1). Five of the non-HIV-infected patients (patients 1, 3, 5, 14, and 16; 29%) and four of the HIV-positive patients (patients 18, 19, 20, and 29; 22%) were positive. In all but one case (patient 16), the gene 63-encoded protein samples had also been positive for RNA for gene 63. Positive staining in both normal and HIV-positive samples was detected predominantly in neurons, with only occasional nonneuronal cells positive, as had been the case for RNA detection. The neuronal labeling pattern was mainly cytoplasmic and only occasionally nuclear (Fig. 1). No labeling was detected in the negative control tissues, while the VZV-infected skin biopsy section was positively labeled.

Temporal pattern of VZV RNA expression in explanted TG and DRG. Having demonstrated an apparently restricted profile of VZV RNA expression in a large number of fixed human ganglia, we wondered whether this represented localized viral reactivation or true viral gene expression in latency. We reasoned that a useful comparison which might shed light on this issue would be with the temporal pattern of VZV gene expression in explanted ganglia, since the latter should be different due to possible viral reactivation in vitro. The combined results of this series of experiments are shown in Table 4. Fresh TG and DRG tissues all from normal subjects were explanted as

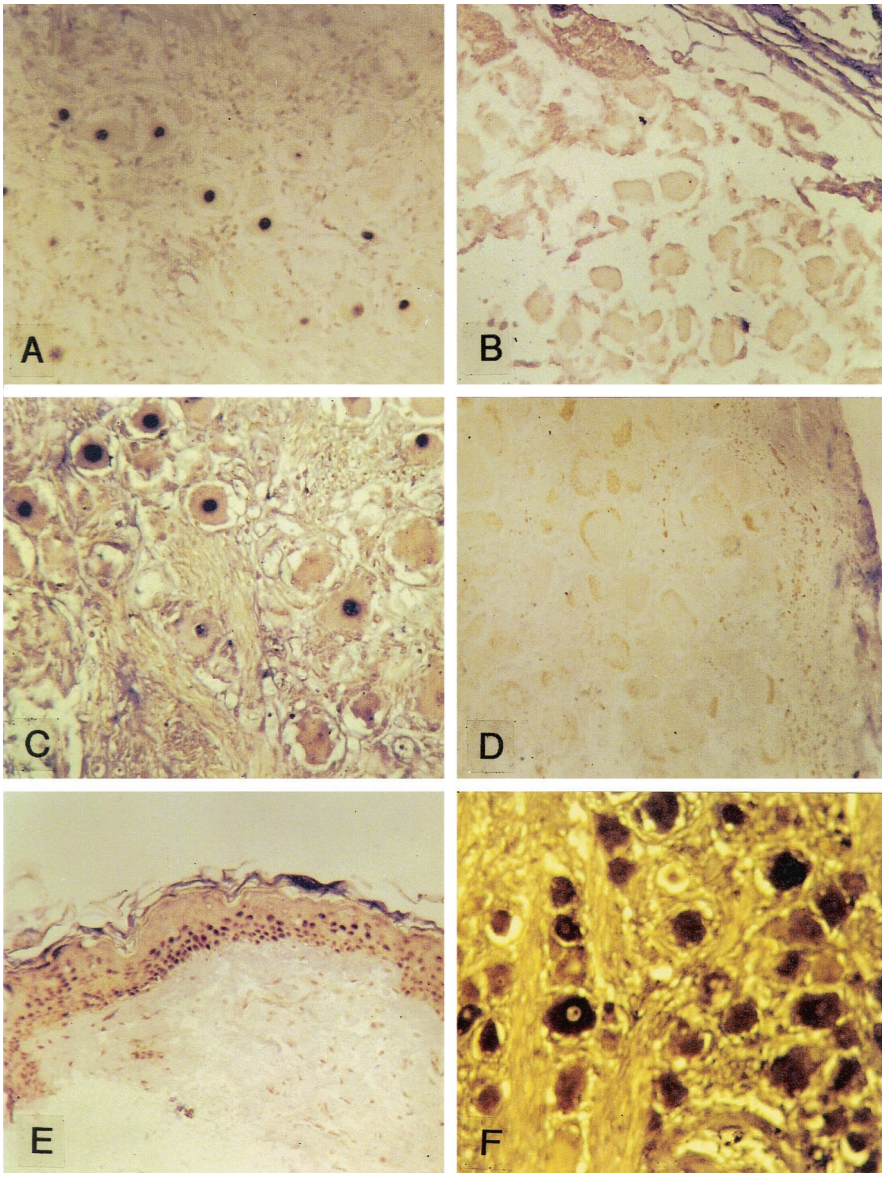

FIG. 1. (A to D) ISH of human TG with DIG-labeled probes. (A) Normal TG hybridized with VZV gene 62 probe. Several neurons are labeled. (B) Control, infant TG hybridized with VZV gene 62 probe. No cells are labeled. (C) HIV-positive TG hybridized with VZV gene 29 probe. Several neurons are labeled. (D) Normal TG hybridized with VZV gene 4 probe. No cells are labeled. (E) Positive control. VZV-infected skin biopsy hybridized with VZV gene probe 62. Many cells are labeled. (F) Immunocytochemical staining of normal TG labeled with polyclonal antibody to VZV gene 63 protein. Many neurons show positive labeling. (Magnification, ×450 in all cases).

soon as possible after death, although in these experiments specific clinical details were not available because of ethical and/or legal restrictions. The genes chosen were putative latency associated (genes 29 and 63) and non-latency associated (genes 18, 28, and 40). In some cases two or three ganglia were available from the same subject (a total of 11 ganglia from five subjects). It can be seen from Table 4 that the pattern was very different from that seen in the 35 prefixed tissues in that all five VZV genes studied were detected in most cases of the explanted ganglia at various times in culture over a period of up to 11 days, which was the maximum time that the tissues remained intact. It is noteworthy that in the ganglia from samples 1 and 2, RNAs for all genes were detected by ISH at the earliest time points (14 to 24 h) after death of the individuals. In this series of in vitro experiments, however, the variability of the assays was somewhat greater than with the fixed ganglia, and so more equivocal results were reported. However, when VZV RNA was detected in explants, the localization was, again, predominantly neuronal. No infectious virus release was detected during the course of these experiments.

DISCUSSION

The increasing clinical significance of VZV infections of the nervous system, especially in immunosuppressed patients, makes it important to understand the molecular mechanisms of VZV latency and reactivation (8, 11). In this study, the most extensive of its kind to date, we provide ISH data for 35 individuals, including 18 who were HIV positive, which define the profile of VZV gene transcription in neurons in latently

TABLE 4. Detection by ISH of VZV RNA in explanted human TG and DRG[a]

Sample	Time (h) after death	Gene 18	Gene 28	Gene 29	Gene 40	Gene 63
1 TG	14	±	+	+	ND	+
	64	+	+	+	ND	+
	168	−	+	ND	+	+
1 DRG	14	±	ND	ND	ND	+
	64	−	ND	ND	ND	−
	168	−	ND	ND	ND	−
2 TG	<24	ND	+	+	ND	−
	264	ND	+	+	ND	±
2 DRG (1)	<24	+	−	+	+	+
	64	−	ND	+	+	−
	264	NON	NON	NON	NON	NON
2 DRG (2)	64	−	+	+	+	+
	264	NON	NON	NON	NON	NON
2 DRG (3)	<24	ND	ND	+	+	+
	64	+	+	+	+	+
	264	−	−	±	+	±
3 TG (1)	48	−	ND	+	ND	−
	120	ND	ND	−	ND	−
	216	ND	ND	−	ND	−
3 TG (2)	48	−	ND	+	ND	+
	216	−	ND	−	ND	ND
4 TG	<24	−	ND	−	ND	−
	120	−	ND	±	ND	−
	192	−	ND	+	ND	−
	264	NON	NON	NON	NON	NON
5 TG (1)	168	+	ND	+	ND	+
	192	−	ND	±	ND	±
5 TG (2)	48	−	ND	+	ND	+
	192	+	ND	+	ND	+

[a] ND, not determined; +, VZV positive; −, VZV negative; ±, equivocal result; NON, no neurons detected.

infected TG. It was found that the most frequently detected VZV transcripts, in both normal and HIV-positive subjects, were those corresponding to genes 21, 29, 62, and 63. This is consistent with previous reports which were based on much smaller sample sizes, some of which used similar techniques (1–4, 19). We have made the assumption that the most frequently detected viral gene transcripts were the most likely to represent truly expressed latency genes. By contrast, we rarely or never detected RNA for VZV genes 28, 40, and 61 in either normal or HIV-positive subjects, strongly suggesting that they are not likely to be latency-associated genes, with their occasional detection possibly representing foci of localized and limited viral reactivation. However, in both normal and HIV-positive subjects, we also detected an intermediate-frequency RNA for VZV genes 4 and 18. While the former is consistent with some (5, 13), but not all (19), previous studies and is probably also latency associated, the finding of nine TG positive for viral RNA for gene 18 was unexpected, as it has not been previously implicated in viral latency, although the extent to which it has been specifically looked for is unclear. VZV gene 18 codes for the small-unit ribonucleotide reductase (HSV homolog UL40) (20), and we are unable at this stage to determine the significance of this observation and whether it represents a truly expressed latency gene or limited viral reactivation in these tissues. The patterns of VZV gene expression in the normal and HIV-positive subjects were very similar, although in most cases the various RNA transcripts were detected more frequently in the HIV-positive patients. This latter finding is consistent with our previous observations which used ISH to detect VZV DNA in normal and HIV-positive TG (12). RNA was detected predominantly in neuronal nuclei in all cases, which is also entirely consistent with our previous studies of VZV DNA localization in human TG (12). There is now a general consensus that latent VZV is located predominantly, if not exclusively, in neurons (18).

In this study we detected VZV gene 63-encoded protein predominantly in neurons in a minority (25%) of TG cases, both HIV positive and normal. Interestingly, RNA for VZV gene 63 was also the most frequently detected viral transcript detected in this study. VZV gene 63 plays a transactivating and transrepressing role in VZV gene regulation and is the homolog of HSV gene ICP22 (10). While this is consistent with some previous reports of both human (17) and rat (6) VZV latency, nevertheless this finding appears not to be a frequent event. It was necessary to screen large numbers of ganglia to detect positive staining for this protein, and the significance of its presence in the minority of ganglia is not known. Nevertheless, our data provide further support for a possibly significant role of gene 63 in the latency process. In one case, gene 63-encoded protein was detected without the corresponding RNA. This presumably reflects technical factors such as assay sensitivity or else a greater abundance of protein than of RNA, which may have been degraded during the procedures. Another possibility is that VZV infection could have been focal and happened to be in the region of antibody staining.

In an attempt to help interpret the ISH findings on the ganglion sections, we carried out a series of experiments to determine whether the pattern of VZV RNA detection in explanted normal TG and DRG cultured soon after death was different from that seen in the prefixed TG. Interestingly, we found that the gene expression profiles were different in the two groups, with a restricted pattern in the fixed ganglia contrasting with the detection of all VZV genes in the explanted ganglia. In addition, the detection of some or all the VZV transcripts in two subjects at very early time points indicates that viral reactivation may have occurred very soon (within 14 h) after death of the individuals. Alternatively, this finding could indicate that these two subjects may have had an active VZV infection at the time of death, although this is probably less likely. Our data showing increased VZV gene expression with time in tissue culture differ from a previous study by Vafai et al. (22), in which late VZV proteins were not detected with monoclonal antibodies after 11 to 17 days in culture. The reasons for this apparent discrepancy are unclear but may relate to different sensitivities of the two methods used. Because of these marked differences in RNA expression in the fixed TG compared with the explanted ganglia, we think it highly likely that most, if not all, of the VZV transcripts detected in the 35 fixed ganglia probably reflected true latent gene expression. However, based on these and our previous studies (12, 13), in which we have emphasized the difficulties and potential pitfalls of applying molecular techniques to fixed human nervous tissues, we have reached the conclusion that complete certainty on the nature of VZV gene expression in these fixed human TG and DRG is not possible to achieve. While the data presented here certainly support the notion of limited and specific VZV gene expression in latently infected human ganglia, it is unlikely that the possibility of prior viral reactivation at or soon after death of the individual can ever be totally excluded. Detailed studies of VZV gene expression in animal models of latent VZV infection will probably be required to circumvent this inherent uncertainty.

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